CONCANAVALIN A INHIBITS THE INTERACTION OF SNAKE VENOM 5'-NUCLEOTIDASE AND ACTIN

H. G. MANNHERZ and M. MAGENER
Max-Planck-Institut für Medizinische Forschung, Abt. Biophysik, Jahnstraße 29, 6900 Heidelberg, FRG

Received 1 April 1979

1. Introduction

Plasma membrane enzyme 5'-nucleotidase has been shown to reactivate DNase I activity of the actin: DNase I complex leading to separation into its protein constituents and to repolymerisation of actin [1,2]. 5'-Nucleotidase exhibits the reactivating effect when isolated from either rat bile or snake venom [1] or possibly when incorporated into liver plasma membranes [2]. Since its enzymatic activity is inhibited by concanavalin A (Con A), both when isolated and membrane bound [3], the effect of Con A on the reactivating effect of 5'-nucleotidase on the actin: DNase I complex and its interaction with skeletal muscle actin was investigated.

The results have indicated that complete inhibition of 5'-nucleotidase by Con A also leads to an inhibition of its ability to interact with actin. The results presented have been published in abstract form [4].

2. Materials and methods

Bovine pancreatic DNase I was a commercial product of Worthington Corp. (cat. no. 2007), 5'-nucleotidase from *Crotalus adamanteous* venom (grade III) and concanavalin A (Grade IV) were obtained from Sigma Corp.

Synthetic actin: DNase I complex was prepared as in [1]. Heavy meromyosin subfragment 1 (HMM-S1) was prepared from rabbit back and leg muscles as in [5] and generously provided by Dr R. S. Goody. Protein concentrations were determined either optically using $E^{1\%}_{280\ nm}$ = 12.3, 7.6, and 11.4 for DNase I, HMM-S1, and Con A, respectively, or by the difference method [7]. Molarity of 5'-nucleotidase was calculated on the basis of mol. wt 70 000 [1,4].

DNase I activity was measured using the hyperchromicity test and 5'-nucleotidase activity was determined optically as in [1]. HMM-S1 ATPase was measured using the linked enzyme optical assay system as in [6]. Enzymatic tests were carried out at 30°C using an Aminco DW 2 spectrophotometer. Hydrolysis of actin-bound [^{3}H]ATP was followed with time by analysis of perchloric acid-quenched samples by thin-layer chromatography (TLC) on polyethylene impregnated cellulose strips as in [6]. [8-^{3}H]ATP was obtained from Amersham Buchler, FRG, DOWEX 1 from Serva, Heidelberg, and adenosine deaminase (EC 3.5.4.4.), lactate dehydrogenase (EC 1.1.1.27.) and pyruvate kinase (EC 2.7.1.40.) from Boehringer, Mannheim. Polyethylene-impregnated cellulose TLC sheets were obtained from Machery and Nagel, Düren and α-methyl-D-mannoside from Serva, Heidelberg. All other reagents were of analytical grade.

All incubation tests including lectins were carried out in 10 mM Hepes buffer (pH 7.2) 10 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM NaN_3 (buffer A) at 37°C.

Abbreviations: Hepes, 2,4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, Tris-(hydroxymethyl)-amino-methane; ATP, adenosine triphosphate; AMP, adenosine monophosphate, NADH, β-nicotinamide-adenine-dinucleotide, reduced form

Nomenclature: DNase I or deoxyribonuclease I, EC 3.1.4.5; 5'-nucleotidase, EC 3.1.3.5 (5'-ribonucleotide phosphohydrolase)

3. Results

The inhibition of the reactivating effect of 5′-nucleotidase on actin : DNase I complex by Con A is illustrated in fig.1, where 10 μM actin : DNase I complex was incubated with 1 μM 5′-nucleotidase in the presence and absence of 1 μM Con A. At these concentration ratios Con A inhibits the enzymatic activity of 1 μM 5′-nucleotidase completely, whereas the DNA degrading activity of 10 μM actin : DNase I complex or 10 μM free DNase I is only slightly reduced (by 2% and 10%, respectively). The extent of reactivation of the actin : DNase I complex by 5′-nucleotidase is clearly inhibited by Con A when followed with time. This inhibition is progressively relieved after addition of increasing concentrations of α-methyl-mannoside (αMM) to the incubation mixture. Addition of 5 mM αMM reverses the inhibitory action of Con A on actin : DNase I complex and isolated DNase I, whereas 5′-nucleotidase activity is still inhibited by 74% (also see legend of fig.1). However, only at αMM > 5 mM a restoration of the reactivating effect of 5′-nucleotidase on actin : DNase I complex is observed.

Since 5′-nucleotidase was found to cosediment with polymeric actin [1] and to accelerate the rate of actin polymerisation as judged by the rate of increase in the intensity of scattered light, the influence of Con A on the interaction of 5′-nucleotidase with monomeric and polymeric actin was investigated. Due to the large increase in turbidity when adding Con A to solutions containing G-actin and 5′-nucleotidase, the rate of actin polymerisation could not be followed optically. Since, however, actin polymerisation is accompanied by the hydrolysis of G-actin-bound ATP to ADP and P_i, the rate of ATP hydrolysis was taken as a measure of actin polymerisation [6]. Figure 2 illustrates the effect of 5′-nucleotidase in the presence and absence of Con A on the rate of ATP hydrolysis by polymerising actin. In these experiments the reaction was started by the addition of 2 μM G-actin having [^{3}H]ATP bound and the extent of ATP hydrolysis was determined from samples taken at the time intervals indicated. Since the actin concentration was chosen to be close to the critical concentration

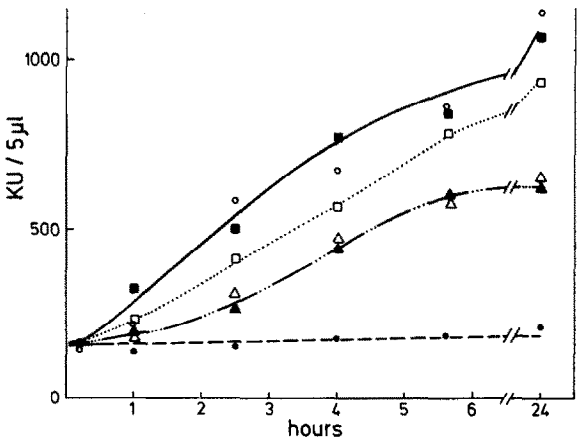

Fig.1. Effect of Con A on the reactivating ability of 5′-nucleotidase on actin : DNase I complex (AD). (●) 10 μM AD incubated in buffer A at 37°C on its own; (○) with 1 μM 5′-nucleotidase; (▲) plus 1 μM Con A; (△) further supplemented with 5 mM αMM; (□) with 10 mM αMM; (■) with 50 mM αMM. DNase I activity in Kunitz units (1 KU = ΔA_{260} of 0.001/min). 5′-Nucleotidase activity after 24 h incubation: (○) 0.53; (▲) 0.01; (△) 0.14; (□) 0.24; (■) 0.36 × 10^{-8} mol AMP/min and 5 μl incubation mixture.

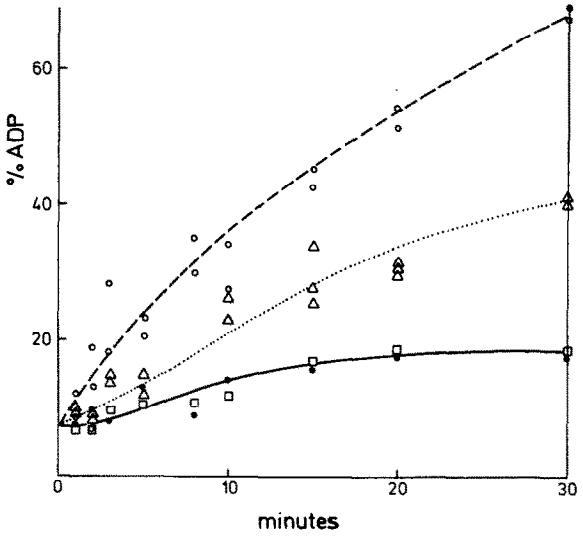

Fig.2. Effect of 5′-nucleotidase in the presence and absence of Con A on the rate of ATP hydrolysis of polymerising actin at 25°C in buffer A plus 0.1 M KCl. Reaction was started by addition of 2 μM G-actin. Immediately before addition G-actin in 5 mM Hepes buffer, (pH 7.4), 0.1 mM $CaCl_2$, 0.5 mM NaN_3 and 0.1 mM ATP supplemented with [^{3}H]ATP was treated with Dowex 1 to remove free ATP. (●) 2 μM actin alone; (□) plus 5 μM Con A; (○) plus 5 μM 5′-nucleotidase; (△) plus 5 μM 5′-nucleotidase and 5 μM Con A. 5′-Nucleotidase used had been treated with 5 mM sodium mersalyl for 1 h at room temperature.

of polymerisation [9], only a small fraction of the total [^{3}H]ATP was hydrolysed at a low rate in the absence of 5′-nucleotidase. In the presence of this enzyme, however, the rate and extent of ATP hydrolysis are both markedly increased paralleling in both cases the rate of increase of the intensity of scattered light when determined separately. Again this effect of 5′-nucleotidase is partially reversed when incubated with an equimolar concentration of Con A (fig.2). It was found that commercial 5′-nucleotidase is contaminated by an ATPase (most probably a Ca^{2+}, Mg^{2+}-ATPase) which could not effectively be removed by the purification procedure in [1]. This ATPase, however, could be completely blocked after 1 h incubation with 5 mM sodium mersalyl without affecting the specific enzymatic and actin : DNase I complex reactivating activity of 5′-nucleotidase. Furthermore, immediately before addition the [^{3}H]ATP–G-actin was treated with Dowex 1 to remove any free ATP. Due to the high binding constant of ATP to G-actin [10] all nucleotide present could then be assumed to be actin bound.

5′-Nucleotidase has been observed to activate the F-actin-activated Mg^{2+}-dependent myosin ATPase also (to be published). Again it was found that addition of Con A reverses this effect as indicated in fig.3 without affecting the acto-HMM-S1 ATPase alone.

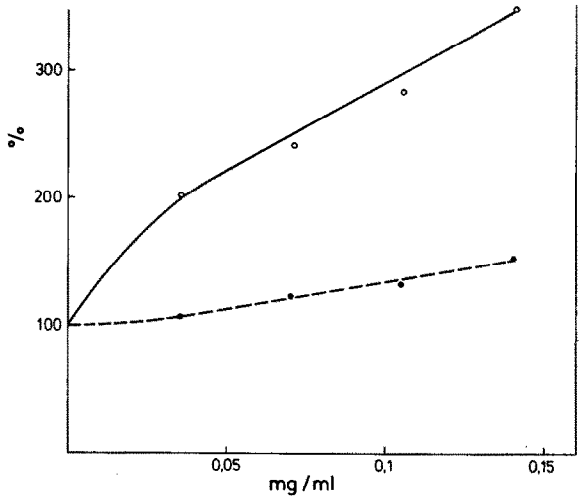

Fig.3. Effect of increasing concentrations of 5′-nucleotidase on the actin-activated HMM-S1-ATPase in the presence and absence of Con A. Assay mixture contained in 1 ml: 100 mM KCl, 2 mM $MgCl_2$, 2 mM phosphoenolpyruvate, 0.8 mM NADH, 0.5 mM ATP, 0.5 mg lactate dehydrogenase, 0.5 mg pyruvate kinase and 50 mM Tris–HCl (pH 8.0), (○) ATPase activity of 75 μg HMM-S1 (A1) and 0.4 mg F-actin plus increasing concentrations of 5′-nucleotidase. (●) plus Con A at a 0.68 weight ratio to 5′-nucleotidase. 100% ATPase activity equals 3.6×10^{-6} mol ATP hydrolysed/min.

4. Discussion

The results presented indicate that the interaction of 5′-nucleotidase with actin is inhibited by the lectin Con A. As indication of the interaction with actin its observable effects on the actin : DNase I complex, actin polymerisation and actomyosin ATPase were investigated. Although snake venom 5′-nucleotidase was used in these experiments the results obtained may be extrapolated to 5′-nucleotidases from other sources, since when isolated from rat bile (to be published) or human placenta (kindly supplied by Dr. W. Gutensohn) these are also able to reactivate the actin : DNase I complex.

The influence of Con A on the enzymatic activity of 5′-nucleotidase has been reported to be biphasic when incorporated into natural membranes with a stimulatory response at low and an inhibitory at high concentrations, whereas for the isolated enzyme only an inhibitory response at high concentrations of Con A is observed [4,8]. In the experiments described an effect of Con A on the interaction of isolated 5′-nucleotidase with actin could only be observed when its enzymatic activity was $\geqslant$ 50% inhibited. The dose response curve of the interaction of 5′-nucleotidase when membrane bound with actin may also be biphasic, since a biphasic response of receptor mobility of intact lymphocytes to Con A binding has been reported [11].

5′-Nucleotidase is an ubiquitous plasma membrane enzyme of eukaryotic cells with its active center facing the cell exterior. Since its enzymatic activity is not altered after incubation with actin or during activation of the actin : DNase I complex, its actin interacting site appears to be distinct from its enzymatic center. Although it is not yet proven that 5′-nucleotidase is an integral membrane protein spanning the whole plasma membrane, it is tempting to speculate that a cytoplasmic part of this enzyme is able to interact with actin, whose affinity to actin can be

regulated by exterior recognition signals like the binding of lectins thus modulating the cellular motile response. This assumption is particularly attractive in view of the finding that Con A receptors cocap with intracellular actin filaments [12].

Acknowledgements

We would like to thank Dr R. S. Goody for critically reading the manuscript and supplying HMM-S1. We also thank Dr W. Gutensohn, München for 5′-nucleotidase from human placenta.

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